Evaluation of dual-acting PIM/PI3K inhibitor IBL-302 in preclinical breast cancer models

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INTRODUCTION

Activating PI3K mutations have been identified in more than 30% of breast cancers. These mutations have been associated with resistance to trastuzumab a HER2-binding monoclonal antibody. PIM kinase expression has been shown to be markedly elevated in PI3K treated breast cancer samples suggesting that it could be a major resistance pathway for PI3K inhibitors in breast cancer, potentially limiting their clinical utility. IBL-302 is a novel molecule that inhibits both PIM and PI3K signalling. This mechanism of action could afford significant benefit in the treatment of breast cancer.

AIMS

The primary objective of this study was to evaluate IBL-302 in preclinical *in-vitro* / *in-vivo* breast cancer models.

STRUCTURE OF IBL-302 – A NOVEL PIM/PI3K INHIBITOR

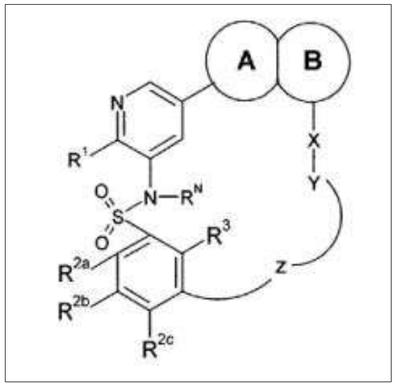
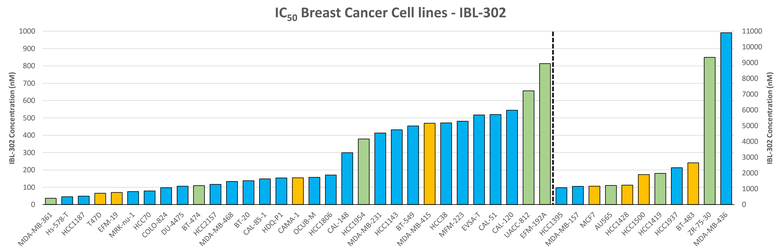


Figure 1: The structure of IBL-302 is a first-in-class oral kinase inhibitor rationally designed to uniquely combine pan-PIM kinase, pan-PI3K and mTOR inhibition in a single agent. IBL-302 has been tested in over 700 cell lines with activity shown across a broad range of tissue types. Initial analysis of this cell line panel identifies PIK3CA mutation, high PIM kinase expression and elevated MYC expression as factors indicating sensitivity to IBL-302, markers which may be supportive in selecting patient subgroups in clinical trials.

METHODS

- Figure 2: Sanger Institute GDSC screening panel of more than 700 different cancer cell lines in a CellTiter Glo anti-proliferation assay (72hr incubation).
- Figure 3: (**A**) T-test comparing HER 2 AMP / ER+ versus TNBC breast cancer cells, focusing on IC_{50} (**B**) T-test comparing Non MYC AMP versus MYC AMP breast cancer cells, focusing on IC_{50} (**C**) Pearson rank correlation used to determine correlative trend observed between IC_{50} and PIM1 expression (**D**) Pearson rank correlation used to determine correlative trend observed between IC_{50} and PIM3 expression.
- Figure 4: Treatment with IBL-302 (50mg/kg) PO for 21-days BT474 (**C**) and HCC1954 (**D**) in xenograft model versus vehicle controls in BALB/c nude mice.
- Figure 5: Proliferation assays were performed with IBL-302 at increasing concentrations with a static Trastuzumab concentration (10µg/ml) across 7 different cells lines (**A** – **G**). Following 5-day incubation, during which control cells attained 80-90% confluence, all media was removed from the plates, and washed once with PBS. Proliferation was measured using the acid phosphatase assay.

IBL-302 IC_{50} IN 40 BREAST CANCER CELL LINES



Breast Cancer Cell Lines

Clinical Subtype	MDA-MB-361	HS-578-T	HCC1187	T47D	EFM-19	MRK-nu-1	HCC70	COLO-824	DU-4475	BT-474	HCC1569	MDA-MB-468	BT-20	CAL-85-1	HDQ-P1	CAMA-1	OCUB-M	HCC1806	CAL-148	HCC1954	MDA-MB-231	HCC1143	BT-549	MDA-MB-415	HCC38	MFM-223	EVSA-T	CAL-51	CAL-120	UACC-812	EFM-192A	HCC1395	MDA-MB-157	MCF7	AU565	HCC1428	HCC1500	HCC1419	HCC1937	BT-483	ZR-75-30	MDA-MB-436
PIM1 Expression	3.6	6.1	4.5	3.8	4.7	3.5	4.7	5.8	4.6	3.3	4.0	5.2	5.4	5.4	6.7	3.3	3.2	5.1	3.7	5.2	4.4	4.4	4.4	3.7	4.5	3.5	3.5	5.5	3.8	3.5	3.4	4.4	3.6	4.3	3.2	3.9	3.2	3.2	4.7	3.2	3.3	3.9
PIM2 Expression	3.2	3.2	3.4	3.6	3.4	3.3	3.0	3.9	3.4	3.3	4.0	3.2	3.0	3.1	2.9	3.2	2.9	3.5	3.1	3.5	3.5	3.2	3.4	3.6	3.4	4.3	3.1	3.3	3.9	3.7	3.1	3.2	3.5	3.1	2.9	3.5	3.5	3.2	3.1	3.4	3.0	3.9
PIM3 Expression	8.2	8.1	7.7	7.7	8.2	6.7	8.3	8.4	8.6	8.7	6.9	7.7	8.3	8.4	8.7	8.9	7.6	8.4	7.1	6.6	7.2	8.4	7.2	8.0	7.6	7.8	7.6	6.7	8.5	9.1	8.6	8.4	7.4	9.0	10.5	7.7	8.6	9.3	7.3	9.8	8.5	8.7
MYC Expression	8.8	10.9	11.0	8.5	11.4	11.4	9.8	12.4	9.4	10.7	12.2	10.8	10.1	10.6	9.7	11.2	9.7	11.8	11.0	11.3	10.8	11.8	11.2	10.1	10.0	11.8	11.3	9.7	11.6	10.3	10.6	10.5	12.0	12.1	11.4	10.2	10.4	6.4	10.3	11.1	11.6	10.0
MYC amplification						+	+	+			+				+		+			+		+				+	+		+		+	+		+	+	+	+	+	+	+	+	+
PTEN loss							+					+				+		+					+	+				+	+													
PI3KCA mutation	+				+	+					+			+			+		+	+										+				+						+		
ER+	+				+	+					+						+							+							+	+			+			+	+		
PR+	+				+	+					+													+							+	+			+			+	+		

Figure 2: IBL-302 was tested in the Sanger Institute GDSC screening panel of more than 700 different cancer cell lines in a CellTiter Glo anti-proliferation assay (72hr incubation). We subdivided a panel of 40 BC cell lines into their clinical subtype; HER 2 AMP (Green), ER+ (Orange) and TNBC (Blue). Each cell line's corresponding PIM expression, MYC expression, MYC amplification, PTEN loss, PI3KCA mutation, ER+ and PR+ status in displayed in the table.

STRATIFYING GROUPS BASED ON IBL-302 IC_{50}

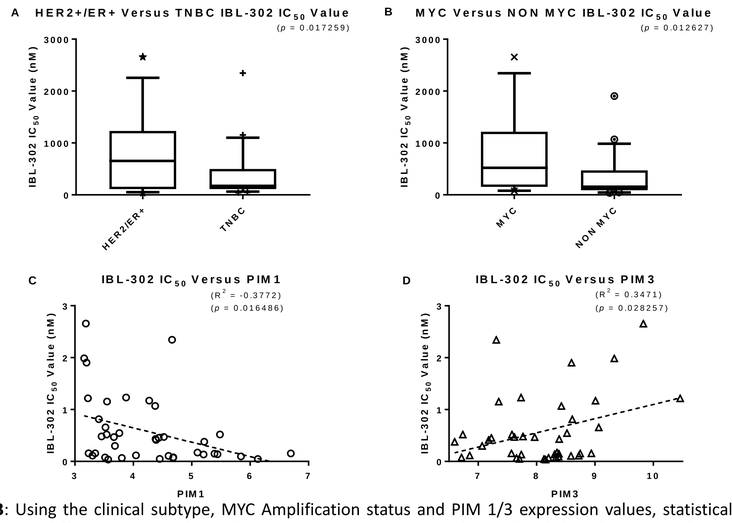


Figure 3: Using the clinical subtype, MYC Amplification status and PIM 1/3 expression values, statistical analysis could stratify different groups when relating to IBL-302 IC_{50}. It was shown that (**A**) triple negative breast cancers had much lower IBL-302 IC_{50s} when compared with HER2 AMP/ER+ breast cancers (p-value = 0.016486). (**B**) MYC amplified breast cancers were shown to have a lower IBL-302 IC_{50} when compared with non MYC amplified breast cancers (p-value = 0.012617). (**C**) Increased PIM 1 expression was shown to correlate with a lower IBL-302 IC_{50} while conversely, (**D**) decreased PIM 3 expression was shown to correlate with a lower IBL-302 IC_{50}.

EFFICACY OF IBL-302 IN BT474/HCC1954 *INVIVO* XENOGRAFT MODELS

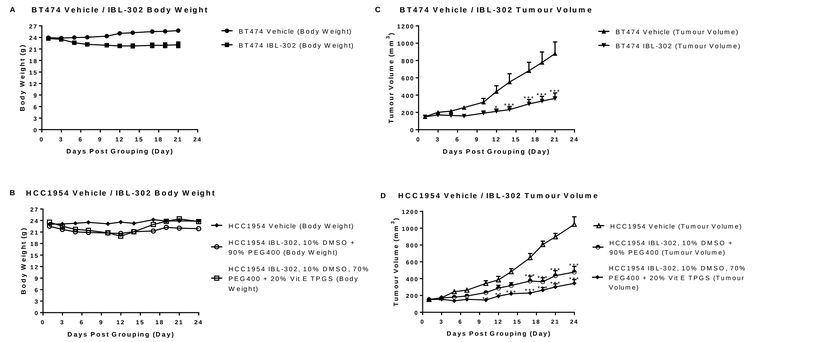


Figure 4: *In vivo* efficacy of IBL-302 was determined in BT474 & HCC1954 subcutaneous xenograft models in BALB/c nude mice. There was no significant change in body weight, in either the BT474 (**A**) or HCC1954 (**B**) xenograft model when compared to the vehicle control. The mean tumour size was significantly decreased in BT474 xenograft model using IBL-302 compared with a vehicle control (**C**). A similar result was seen with a significant decrease in HCC1954 xenograft mean tumour size compared to the vehicle control (**D**).

IBL-302 EFFECT IN TRAS/LAP RESISTANT CELL LINES

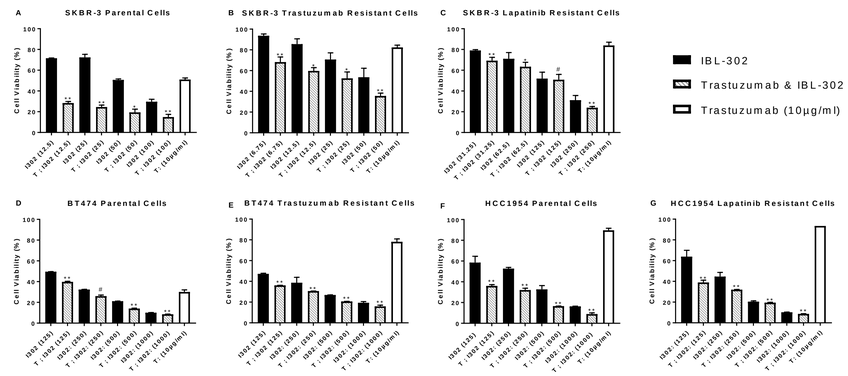


Figure 5: Alkaline phosphatase assay showing the viability of SKBR-3 Parental (**A**), SKBR-3 Trastuzumab resistant (**B**), SKBR-3 Lapatinib resistant cells (**C**), BT474 Parental (**D**), BT474 Trastuzumab resistant (**E**), HCC1954 Parental (**F**) & HCC1954 Lapatinib resistant (**G**) cells after combination of ascending amounts of IBL-302 with a static amount of Trastuzumab (10µg/ml).

CONCLUSION

In conclusion, we have evaluated the preclinical activity of IBL-302 in a range of breast cancer models.

- IBL-302 has shown significant efficacy in a number of breast cancer cell lines *in vitro* and *in vivo*.
- We have stratified and identified a number of factors associated with increased IBL-302 sensitivity through statistical analysis (TNBC, MYC amplification & Increased PIM1 expression).
- Initial statistical analysis suggests IBL-302 could be effective in TNBC groups.
- In combination with Trastuzumab, IBL-302 demonstrated the best anti-proliferative effects in parental lines and in SKBR-3 trastuzumab resistant cell lines with acquired resistance to Trastuzumab.
- Our results demonstrate the preclinical efficacy of IBL-302 as a single agent and in combination with trastuzumab in models of HER2-positive breast cancer. We believe that further studies assessing the impact of IBL-302 with novel HER2-targeted agents are warranted.

ACKNOWLEDGEMENTS

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